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Ob-Stopping Obesity, Metabolic and Immune-Mediated Disorders

Giuseppe Matarese[1,2,*] and Veronica De Rosa[1,*]
[1]Laboratorio di Immunologia, Istituto di Endocrinologia e Oncologia Sperimentale, Consiglio Nazionale delle Ricerche (IEOS-CNR), Dipartimento di Biologia e Patologia Cellulare e Molecolare, Universitá di Napoli "Federico II", Napoli 80131, Italy
[2]Dipartimento di Medicina e Chirurgia, Università degli Studi di Salerno, Baronissi Campus, Salerno 84081, Italy
*Correspondence: gmatarese@unisa.it (G.M.), veronica.derosa@cnr.it (V.D.R.)
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Despite its physiological importance, no part of human leptin receptor (ObR) has been structurally characterized before. In this issue of *Structure*, Carpenter et al. report the crystal structure of the leptin-binding domain of human ObR in complex with the Fab fragment of an ObR-blocking monoclonal antibody (9F8 mAb).

The discovery of leptin (*ob* gene), an adipose tissue-derived cytokine-like hormone, led the scientific community to focus attention on its role as an anorexic hormone involved in the negative regulation of food intake (Friedman and Halaas, 1998). Leptin is now known to participate in a wide range of biological functions that include—in addition to its function as an adipostat—glucose metabolism, $CD4^+$ T lymphocyte proliferation, cytokine secretion, phagocytosis, regulation of the hypothalamic-pituitary-adrenal axis, reproduction, angiogenesis and cardiovascular pathology, bone formation, and apoptosis (La Cava and Matarese, 2004) (Figure 1). It is now well documented that leptin acts like a cytokine hormone with many pleiotropic effects, as well as that many effects of leptin are acquired through systemic and peripheral activities.

Leptin is a helical-cytokine structurally similarly to interleukin (IL)-6, IL-12, and IL-15. Its receptor (ObR) belongs to the class I cytokine receptors, which includes gp-130, the common signal transducing component for the IL-6-related family of cytokines (Tartaglia, 1997). Leptin is expressed mostly in the adipose tissue and at lower levels in the muscle, stomach, and placenta (Friedman and Halaas, 1998). More recently, it has also been shown that leptin can be expressed by activated inflammatory T helper 1 lymphocytes in experimental autoimmune encephalomyelitis, an animal model of multiple sclerosis (Sanna et al., 2003). Accordingly, ObR can be found not only in the hypothalamus and adipose tissue, but also on immune cells such as T lymphocytes and monocytes, endothelial cells, and $CD34^+$ bone marrow precursors (Figure 1).

To date, despite the extensive genetic and cellular analysis, structural studies of ObR were missing. In this issue of *Structure*, Carpenter et al. (2012) elegantly characterize the crystal structure of the Fab fragment of an anti-hObR monoclonal antibody (9F8 mAb) (Fazeli et al., 2006), both in its uncomplexed state and bound to the leptin-binding domain (LBD) of human ObR. Strikingly, 9F8 mAb is the only anti-human ObR neutralizing mAb described to date. The authors crystallized 9F8 mAb in its uncomplexed state and solved its crystal structure at a 2.3 Å resolution. They further solved the structure of 9F8 Fab complexed with LBD at 1.95 Å resolution using Fab-mediated crystallization. Their study describes the structure of the LBD-9F8 Fab complex and the changes induced in 9F8 Fab by LBD binding. The authors also constructed and characterized a molecular docking model of the leptin-LBD complex, which revealed how 9F8 Fab can antagonize leptin signaling. Overall, these findings provide new insight into the mechanism of leptin binding to LBD and the mechanism of 9F8 antagonism of leptin signaling.

To date, the crystallization of isolated LBD or a leptin-LBD complex has proven difficult. Although there are numerous possible reasons for this, a major factor was likely the presence of several flexible loops within LBD, which limit the surface area amenable to crystal contact formation. Fab-mediated crystallization is a powerful tool to improve crystallization of challenging proteins by stabilizing dynamic regions, thus increasing the hydrophilic surface area available for crystal lattice formation and masking unfavorable regions of the protein. Here,

LBD was successfully crystallized. In the future, the use of non-neutralizing antibodies that bind either leptin or LBD may facilitate crystallization of the leptin-LBD complex.

This work provides new information at multiple levels. First, 9F8 mAb binds within the isolated LBD, with a similar affinity to leptin, and their binding is mutually exclusive. Second, 9F8 Fab was crystallized in both its uncomplexed and receptor bound forms, and changes in 9F8 induced by LBD binding were characterized. 9F8 mAb appears to be a good template for structure-based design of a potent leptin antagonist with strong therapeutic potential. Third, because it is predicted that the epitopes of leptin and 9F8 Fab overlap by only 10%, 9F8 Fab can represent a useful tool to mediate co-crystallization of LBD with potential peptide or small molecule drug candidates. This first report of a crystal structure of the ObR can thus facilitate the design of therapeutics modulating leptin signaling.

Though the discovery of leptin energized the study of energy balance, much of the initial enthusiasm waned with the realization that obesity is not a condition of leptin insufficiency but rather of leptin resistance (Coll et al., 2007). Leptin resistance is often described as a state in which circulating levels are elevated with concomitant hyperphagia and obesity. By this standard, most obese individuals are leptin resistant. From a molecular standpoint, leptin resistance is a failure of leptin to activate key signaling molecules in target neurons. Although, leptin activates multiple intracellular signaling molecules whose resistance is often only demonstrated for a few. There is also evidence for variations in leptin-activated STAT-3 in the brain so that leptin sensitivity may vary even within the same individual. What is known about leptin resistance is that it involves at least two separate mechanisms: the first being a reduced transport across the blood brain barrier, and the second being a reduced capacity for intracellular signaling within target neurons (Coll et al., 2007) (Figure 1).

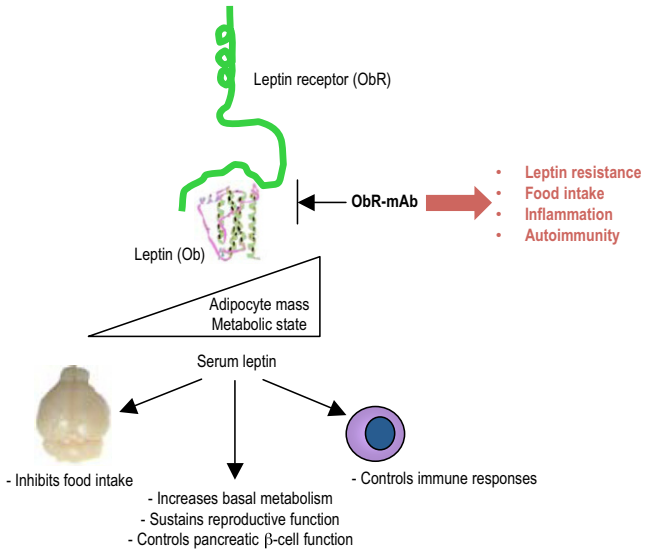

Figure 1. Pleiotropic Effects of Leptin and of ObR Neutralization
The figure shows that leptin induces pleiotropic effects through activation of its receptor at central and peripheral levels, such as control of food intake, increase of basal metabolism, and control of reproductive, pancreatic, and pro-inflammatory immune responses (La Cava and Matarese, 2004). ObR blockade with 9F8 could be utilized to control metabolic and immune dysregulation. These combined approaches could be utilized to dampen leptin resistance, inflammation, and autoimmunity though direct neutralization of the leptin axis.

A reduction in leptin transport across the blood brain barrier has been demonstrated in obese animals. Additional work has documented a reduced sensitivity to peripheral leptin signaling prior to loss of central leptin sensitivity, yet it has not been demonstrated that alterations in leptin transport directly influence body weight or food intake in lean or obese animals. As such, the role of reduced transport in the etiology of obesity is not fully clear (Coll et al., 2007). Basing on these considerations, a possibility should exist to reverse leptin resistance in obesity by using anti-ObR neutralizing antibodies, such as 9F8 mAb, followed by administration of recombinant leptin. This novel strategy would be based on the possibility that ObR neutralization with antibodies could desensitize the ObR, and the subsequent administration of recombinant leptin would inhibit food intake—more efficiently than using only leptin administration. ObR neutralization could also represent an alternative molecular tool in the treatment of autoimmunity and inflammation (De Rosa et al., 2006) (Figure 1). Indeed, ObR blockade reduces the activation of effector T cells and to induce expansion in vitro of regulatory T (Tregs), an immune cell subset responsible for maintenance of immune tolerance (De Rosa et al., 2007). This effect could be exploited to expand ex-vivo Tregs from autoimmune patients in which a specific defect in Treg number has been observed. Another approach could be the use of ObR neutralization in vivo for the treatment of inflammation, although this would generate concerns related to the fact that since ObR short forms are expressed in many tissues, neutralizing monoclonals against the extracellular domain could bind in many tissues and determine potential tissue damage via complement activation. The generation of Fab fragments, such as in the case of 9F8, could possibly reduce these adverse effects.

To conclude, there are still many questions about the role of many molecules at the interface between metabolism and immunity in the regulation of the two systems. While new information is unveiling the complex connection between metabolism and immunity, further research is needed. Yet, the adipose tissue can no longer be regarded merely as a store of body fat, but rather as an active participant in the regulation of essential body processes with prominent roles, particularly in the balance of inflammation and immune homeostasis. In this context, antagonism of the leptin axis could represent a novel strategy to control inflammation and peripheral leptin resistance in obesity and related conditions, to possibly develop novel therapeutic strategies to treat obesity, metabolic disorders, and immune dysregulation.

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The 19S Cap Puzzle: A New Jigsaw Piece

Eva M. Huber[1] and Michael Groll[1,*]
[1]Center for Integrated Protein Science, Department Chemie, Lehrstuhl für Biochemie, Technische Universität München, Garching D-85747, Germany
*Correspondence: michael.groll@ch.tum.de
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After elucidation of the atomic details of 20S proteasomes, current research focuses on the regulatory 19S particle. In this issue of *Structure*, He et al. present the crystal structure of Rpn2 and use electron microscopy to examine differences between Rpn2 and Rpn1.

Peptide bond hydrolysis constitutes an essential intracellular process, enabling amino acid recycling as well as control of signaling cascades. Cytosolic protein degradation is predominantly mediated by the proteasome, a multicatalytic protease, consisting of 28 subunits that are stacked in four seven-membered rings. Whereas the α subunits form the outer rings of the barrel-shaped 20S core particle (CP), the inner rings are exclusively built of β subunits which exert proteolytic activity. Archaeal CPs bear only one type of α− and β subunits and thus harbor seven identical active β subunits. In contrast, eukaryotes incorporate seven distinct α− and β subunits in their CPs, but only three out of the seven β subunits are capable of proteolysis. Apart from the constitutive proteasome, mammals express two specialized versions of CPs, namely the immuno- and the thymoproteasome, which differ in their set of catalytically active β subunits and thus in their biological function.

During the last 15 years, the atomic structure of CPs from different species has been analyzed by X-ray crystallography and, only recently, this collection has been completed by the crystal structure of the mouse immunoproteasome (Huber et al., 2012). Additionally, ATP-independent regulators of this destructive machinery, namely 11S complexes and Blm10, have been structurally investigated. Sitting on top of the CP, the 11S particle, a heptamer of helical monomers arranged around a central pore, induces conformational changes in the N-termini of the α subunits, which ultimately results in the opening of the entry gate to the proteolytic chamber and provides access for substrates (Whitby et al., 2000). In contrast to 11S particles, the atomic structure of Blm10 is indicative of inhibitory rather than stimulatory effects on proteasomal activity (Sadre-Bazzaz et al., 2010).

Despite a number of electron microscopy studies on the most prominent regulatory particle (e.g., da Fonseca and Morris, 2008), the 19S complex, composed of at least 18 different polypeptides, has not yet been characterized at atomic resolution. As an ATP-dependent complex, the 19S cap of CPs plays a key role in the recognition of ubiquitylated substrates and in their unfolding and translocation into the proteolytic chamber. Recent advance in this field is provided by an impressive study in which the 19S particle has been reconstituted in *Escherichia coli* in order to map the individual non-ATPase subunits, termed "Rpn," and the ATPase subunits ("Rpt") by electron microscopy (Lander et al., 2012). Owing to the complex architecture of the 19S cap, a crystallographic approach is quite challenging and, hence, current research focuses on the elucidation of the X-ray structures of single subunits. In this regard, this issue of *Structure* reports the crystal structure of one of the largest 19S subunits, namely Rpn2, solved by He et al. (2012).

Rpn2 adopts a cylindrical shape consisting of two layers of α helices that are wrapped around each other. At the N- and C terminus Rpn2 forms an elongated α-helical segment and a globular domain, respectively, which come close to each other in the tertiary structure. The toroid-like fold of Rpn2 results from a repetitive primary sequence of 35–40 amino acids called proteasome/cyclosome (PC) motif (Kajava, 2002). Remarkably, the 32 HEAT repeats of Blm10 adopt a similar superhelical dome-like structure, indicating that PC and HEAT